

## Antimigratory Evaluation from Curcumin-Derived Synthetic Compounds PGV-1 and CCA-1.1 on HCC1954 and MDA-MB-231 Cells

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### Abstract

Earlier findings reported the anticancer-mediated activities of curcumin-modified compounds Pentagamavunone-1 (PGV-1) and Chemoprevention Curcumin Analog 1.1 (CCA-1.1) with several mechanisms including cell cycle arrest, reactive oxygen species (ROS) production, and cell migration disruption. Our study aims to evaluate the antimigratory activity of PGV-1 and CCA-1.1 on aggressive breast cancer cell lines (MDA-MB-231 and HCC1954 cells) and their effect on HER2 protein. The trypan blue exclusion method was conducted for the antiproliferative effect. The PGV-1 or CCA-1.1 effect on cell migration was determined by wound healing assay. Using gelatin zymography, we checked the secretion level of matrix metalloproteinase (MMP). We also evaluated the human epidermal growth receptor-2 (HER2) level after incubation with PGV-1 or CCA-1.1 in HCC1954 cells by western blot. Based on the antiproliferation assay, MDA-MB-231 and HCC1954 cells were sensitive to PGV-1 and CCA-1.1. MMP-2 was only observed in HCC1954 cells while MMP-9 was only observed in MDA-MB-231. Both PGV-1 and CCA-1.1 significantly suppressed MMP-9 activity in MDA-MB-231 cells. Moreover, PGV-1 inhibited HER2 protein levels in HCC1954 although it was not significant, whereas CCA-1.1 did not affect HER2 protein. This study strengthens the scientific evidence for PGV-1 and CCA-1.1 activities for future exploration as candidate chemotherapy with multitarget against breast cancer.

**Keywords:** Curcumin analog, cell migration, MMP-9, HER2, breast cancer.

### INTRODUCTION

Breast cancer is driven by high heterogeneity, as reflected in the classification of subtypes based on several indicators such as hormone receptors, human epidermal growth factor, and proliferation biomarkers (Turashvili and Brogi, 2017). For instance, the cells presented with positive immunohistochemical detection for es-

rogen receptor and/or progesterone receptor occurred by 70%, while the other subsets such

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as human epidermal growth factor receptor 2 (HER2)-positive and triple-negative breast cancer (TNBC) cases that constituted around 15%–25% in invasive breast cancer (Fragomeni, *et al.*, 2018). Despite the lower frequency, the amplification of HER2 enhances abnormal tumor growth and fosters malignant phenotype (Freudenberg, *et al.*, 2009) as HER2 activation promotes cell proliferation via the RAS/MAPK pathway (Sangrar, *et al.*, 2015). The patient with TNBC elevates the risk of metastasis considering the tumor cells' aggressivity and the lack of usual biomarkers (Rakha and Chan, 2011), making it more difficult during therapy. Matrix metalloproteinase (MMP) is known for its pivotal role in metastasis with its capability to degrade the extracellular matrix (ECM) and permits cancer cells to migrate (Lv, *et al.*, 2018). The MMP-2 and MMP-9 are most well-analyzed in breast cancer cells (Li, *et al.*, 2017; Zhang, *et al.*, 2014), and serve as common biomarkers for cell migration.

The curcumin derivative compound, Pentagamavunone-1 (PGV-1) (Figure 1A) and recent developed Chemoprevention Curcumin Analog 1.1 (CCA-1.1) (Figure 1B) demonstrate specific anti-cancer activities in cancer cells and breast tumor (Meiyanto, *et al.*, 2019, 2021; Novitasari, *et al.*, 2021b, 2021c; Wulandari, *et al.*, 2020). Those curcumin analogs mediate cell cycle arrest in G2/M to inhibit tumor proliferation and elevate reactive oxygen species (ROS) generation to trigger cellular senescence. Furthermore, in molecular docking studies, CCA-1.1 interacts similarly with several PGV-1 targets (Utomo, *et al.*, 2022).

The present study evaluates whether PGV-1 or CCA-1.1 affects cell migration and MMPs secretion using endogenous HER2-positive and a TNBC cell line. A prior study with a similar approach used murine TNBC 4T1 cells and MCF7 cells transfected with the HER2 gene (MCF7/HER2). These compounds inhibit MMP-9 expression and secretion into ECM (Meiyanto, *et al.*, 2021, 2019; Novitasari, *et al.*, 2021a). This study utilizes human

cancer cell lines: the TNBC MDA-MB-231 cells and HER2-endogenous HCC1954 cells. Moreover, this study evaluates the effect of CCA-1.1 and PGV-1 on HER2 protein levels. This study shows that albeit with only one functional group between CCA-1.1 and PGV-1, each compound exhibits a different effect in inhibiting cell migration and secretion of MMP. This study's results support the earlier findings for future development as a candidate for chemotherapeutic agents with multiple targets against breast cancer.

## MATERIALS AND METHODS

### Materials

Pentagamavunone-1 (PGV-1) and Chemoprevention Curcumin Analog-1.1 (CCA-1.1) were procured from the Cancer Chemoprevention Research Center (CCRC), Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia.

The immortalized human HER2-positive HCC1954 cells (CRL-2338) and TNBC MDA-MB-231 cells (HTB-26) were purchased from ATCC® (USA). The HCC1954 cells were maintained in Roswell Park Memorial Institute or RPMI 1640 medium (Wako, Japan), while MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose (Wako, Japan); both were added with 10% fetal calf serum or FCS (Hyclone, USA) and 1x penicillin-streptomycin (Wako, Japan).

### Cell Viability Assay

Approximately  $5 \times 10^4$  cells were seeded into a 24-well plate (Corning, USA) and grown for 24 h. Once the cells were already attached, PGV-1 or CCA-1.1 concentrations (0, 0.5, 1.0, 2.5, 5, and 10  $\mu$ M) were diluted in a culture medium and incubated for 24 and 48 h. Cells were harvested with trypsin-EDTA (Wako, Japan). After the enzymatic reaction was inactivated with a culture medium, 10  $\mu$ L of cell suspension was mixed with 10

$\mu$ L of 0.4% trypan blue (Wako, Japan) and placed into a hemocytometer and directly counted for unstained (identified as viable) cells under the light microscope (Olympus). The viable cells were converted into a percentage of cell viability (from the untreated well), then calculated for a 50% growth inhibitory ( $GI_{50}$ ) value and plotted using Microsoft Excel 2019.

### Migration Assay

A total of  $5 \times 10^4$  cells (in 70  $\mu$ L of medium) were put into each side of Insert Cell Culture (ibidi®, Germany) and incubated for around 24 h. The medium was removed and replaced with the starvation medium consisting of 0.5% FCS and incubated again for 18 h. The next day the starvation medium was replaced with 10  $\mu$ g/mL Mitomycin C (Wako, Japan) diluted in starvation medium and stored for 2 h before being treated with the compound (as explained in Di, *et al.* (2015) and Kolditz, *et al.* (2014)). Right after treatment, the cells were documented under the microscope as 0 h. At the indicated times (24, 42, and 48 h), the cell migration was monitored to analyze the gap closure rate further using ImageJ (version 1.52) (Novitasari, *et al.*, 2018).

### Gelatin Zymography

Cells treated with PGV-1 or CCA-1 in the starvation medium for 24 h were collected for the medium. The medium was centrifuged (15,000 rpm; 10 minutes; 4°C), separated from the cell debris, and used for the assays. Before the experiment, the total protein was determined through the Bradford method. The lysate (with the same total amount of protein for each group) was homogenized in a 5 $\times$  sample buffer before running under SDS-PAGE, impregnated with 1% gelatin. After electrophoresis, the gel was washed in 2.5% Triton X-100 solution for 30 minutes before incubation with a developing buffer (contained 0.05 M CaCl<sub>2</sub>) for 18 h. The next day, the buffer was decanted, and the gel was stained with 0.05% Coomassie brilliant

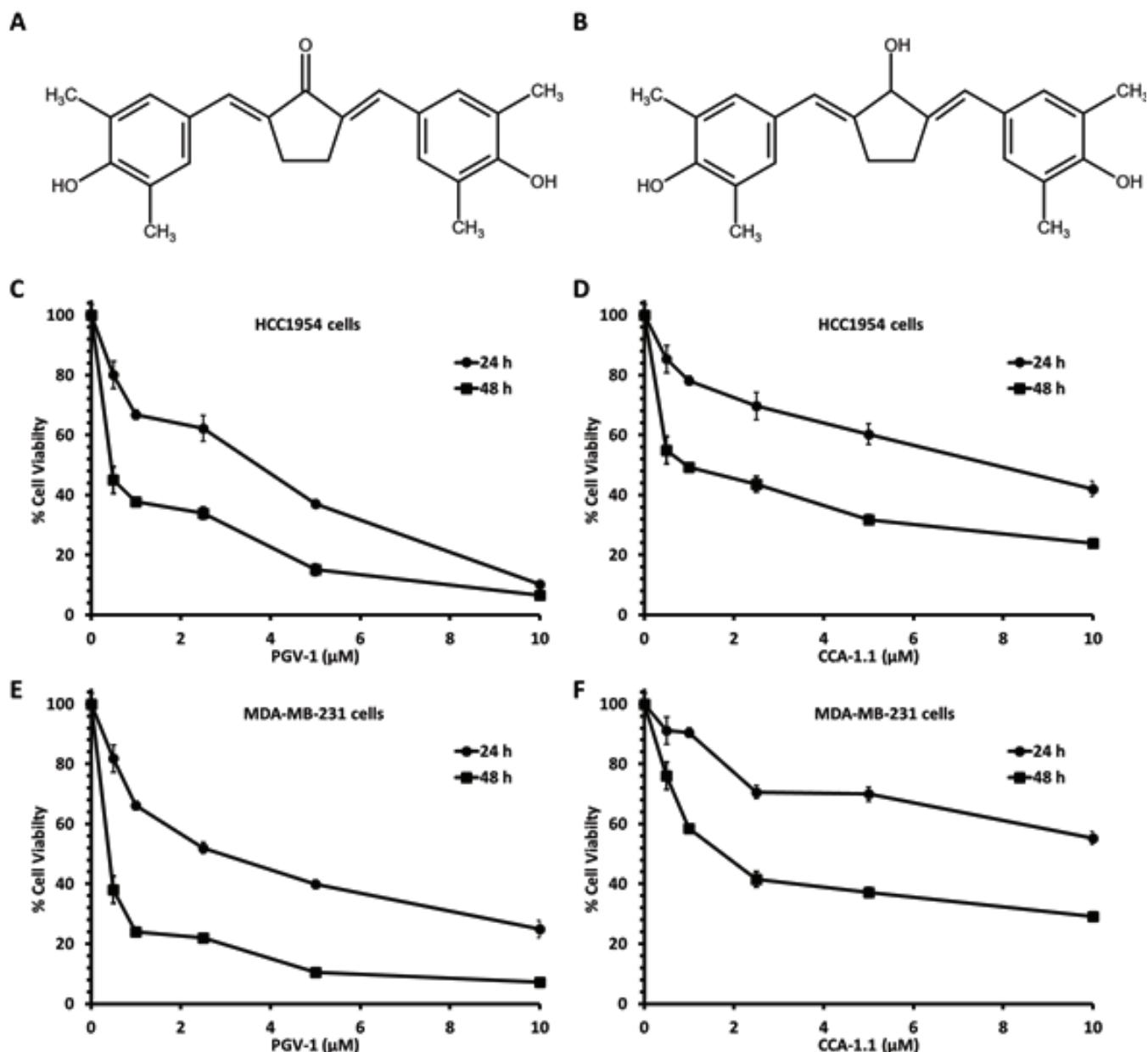
blue for 30 minutes. The gel was washed with a de-staining solution (consisting of methanol and acetic acid) several times until the transparent gel from MMP activity was shown. The gel was scanned as the image for further quantification of the intensity of MMP using ImageJ (version 1.52, Bethesda, MD, USA) (Novitasari, *et al.*, 2021a).

### Western Blot

HCC1954 cells were treated with 2  $\mu$ M PGV-1 or 2  $\mu$ M CCA-1.1 for 24 h and prepared in lysis buffer (contained protease inhibitors) before homogenized with 5 $\times$  sample buffer and loaded into acrylamide-embedded SDS gel for electrophoresis. After the protein marker reached the end of the gel, the electrophoresis was stopped. The gel was blotted into the PVDF membrane before being incubated with HER2 primary antibody from rabbit (Cell Signaling Technology®, #4290, Danvers, MA, USA) overnight (4°C with slow agitation). The mouse-originated  $\beta$ -actin antibody (Cell Signaling Technology®, #3700) was applied as the loading control protein. The next day, the membrane was washed with PBST before incubation with the sheep anti-mouse IgG secondary antibody (Cytiva®, NA931V, HP7 9NA Little Chalfont, UK) or protein A HRP-linked antibody (Cytiva®, NA9120V) for 1 h, then rinsed in PBST before detection of the interest signal with Amersham Enhanced chemiluminescence (ECL™) (Cytiva®) and developed in X-ray film. The film was documented as the image for further quantification of the intensity of HER2 and  $\beta$ -actin through ImageJ.

### The Statistical Analysis

Each data was represented as an average from three data, while the error bar was shown as standard error (SE) or standard deviation (SD). The statistical analysis was generated using a one-way analysis of variance (ANOVA) in R studio 1.3 (RStudio, PBC, Boston, MA, USA). The significance value was indicated as mentioned in the figure description.



**Figure 1. Curcumin analogs PGV-1 and CCA-1.1 inhibit cell growth in HCC1954 and MDA-MB-231 cells.** The chemical structure from (A) Pentagamavunone-1 (PGV-1) and (B) Chemoprevention Curcumin Analog 1.1 (CCA-1.1) were drawn using MarvinJS. The antiproliferative curve of (C) PGV-1, (D) CCA-1.1 on HCC1954 cells, (E) PGV-1, and (F) CCA-1.1 on MDA-MB-231 cells at 24 and 48 h incubation. This study used the trypan blue exclusion assay to determine the cell viability described in the methods. The data is displayed as the average of 3 data  $\pm$ SE.

**Table 1. The  $GI_{50}$  score of PGV-1 and CCA-1.1 in HCC1954 and MDA-MB-231 cells.**

| Compound | $GI_{50}$ value ( $\mu M$ ) |      |                  |      |
|----------|-----------------------------|------|------------------|------|
|          | HCC1954 cells               |      | MDA-MB-231 cells |      |
|          | 24 h                        | 48 h | 24 h             | 48 h |
| PGV-1    | 3.5                         | 0.3  | 2.8              | 0.2  |
| CCA-1.1  | 7.4                         | 0.9  | >10              | 1.5  |

## RESULTS

### Curcumin Analogs PGV-1 and CCA-1.1 Suppress the Growth of the HER2-Positive and TNBC Cells

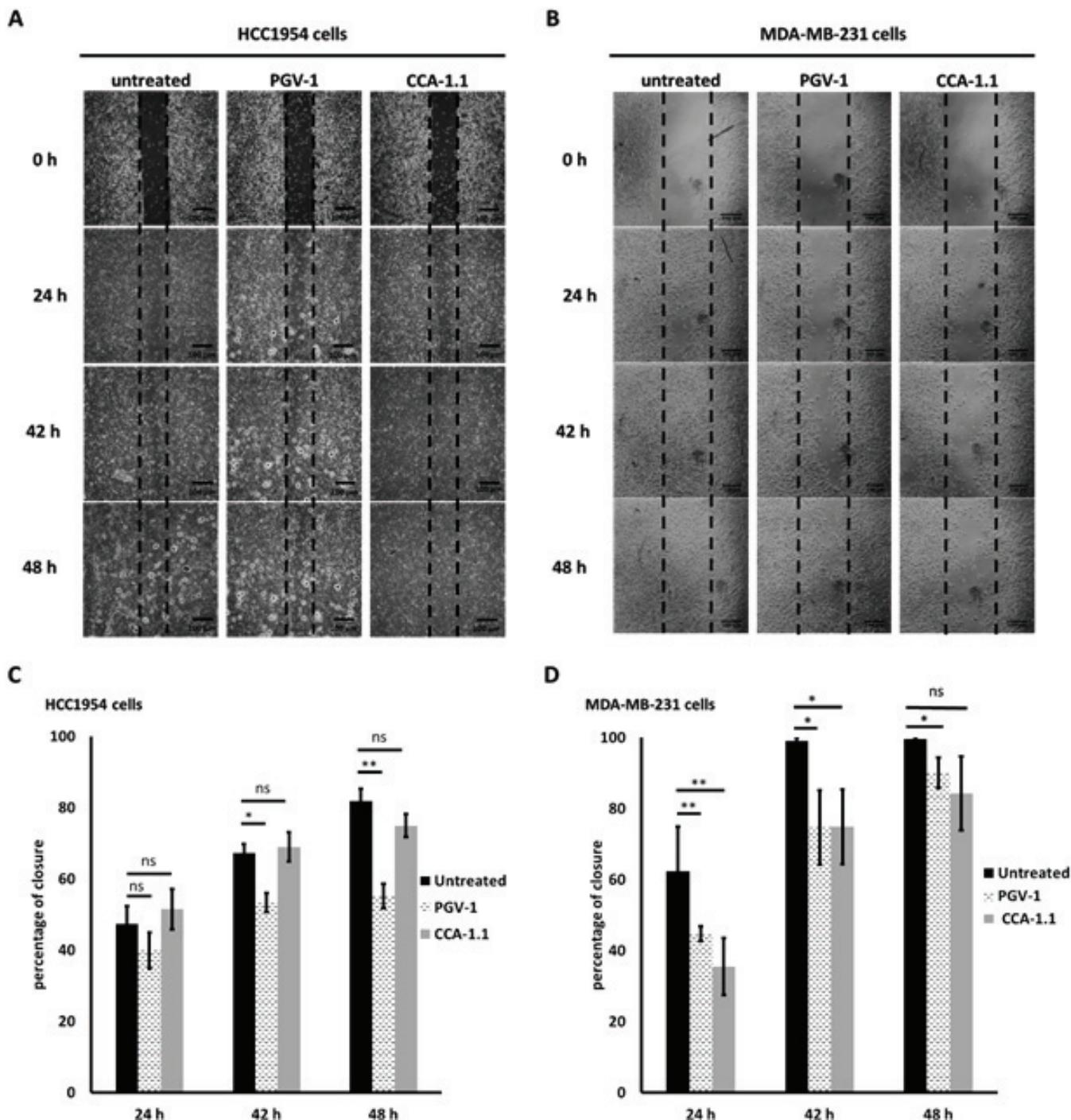
This study evaluates the anticancer properties of PGV-1 and CCA-1.1 in the aspect of migratory activities using endogenous HER2-amplified HCC1954 and TNBC MDA-MB-231 cells. We determined the antiproliferative effect of these compounds during 24 and 48 h of incubation and counted for the viable cells using the trypan blue exclusion method. PGV-1 decreased the proliferation of HCC1954 cells at 10  $\mu M$  during the first 24 h. Upon prolonged incubation to 48 h, even the lowest tested dose of PGV-1 (0.5  $\mu M$ ) resulted in less than 50% of viable cells (Figure 1C). A slightly different pattern occurred in treatment with CCA-1.1, in which during 24 h, the highest concentration reduced 50% of growth; in more extended incubation treatment, the growth inhibitory effect was eventually seen (Figure 1D). The antiproliferation effect of the compound on MDA-MB-231 cells was similar to its effect on HCC1954 cells. PGV-1 inhibited cell proliferation after 48 h (Figure 1E), followed by the impact in CCA-1.1 treated cells which also decreased the cell viability against MDA-MB-231 (Figure 1F). Table 1 presented the 50% growth inhibitory ( $GI_{50}$ ) values. These findings indicated that PGV-1 and CCA-1.1 inhibit breast cancer cell proliferation. Furthermore, PGV-1 was more potent than CCA 1.1 in suppressing both cells' growth.

### The Antimigratory Activity of PGV-1 and CCA-1.1 in HER2-Positive and TNBC Cells

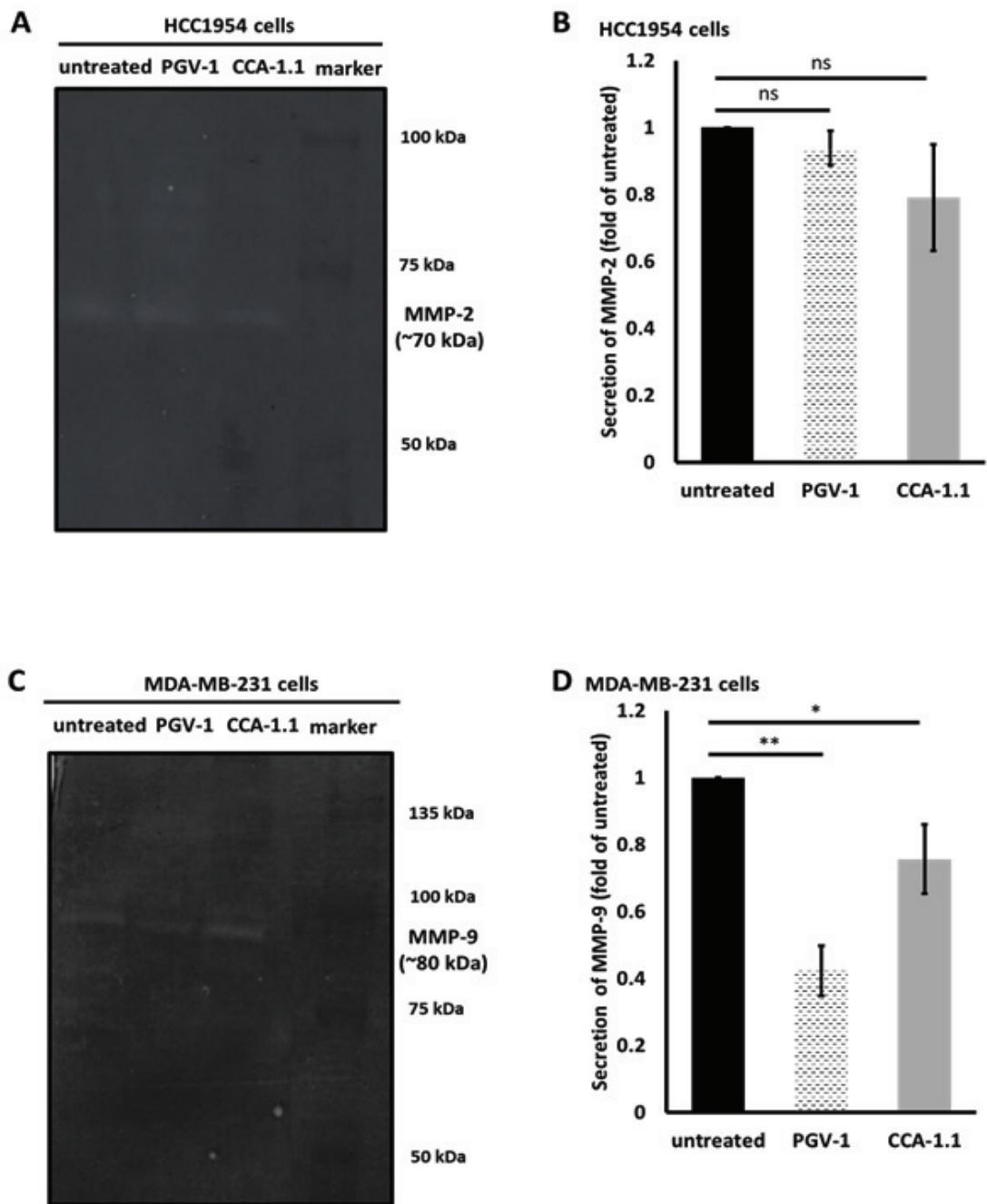
We utilized the wound healing assay to observe the effect of curcumin analogs (PGV-1 or CCA-1.1) in cell migration. We used a serum starvation medium and pretreated the cells with Mitomycin C to inhibit DNA synthesis and minimize the capability of cells to proliferate during the assay (Grada, *et al.*, 2017). The cells were treated with samples at 1  $\mu M$  because at this concentration, the percentage of viable cells was 75-80%; therefore the migration activity of the cells could be observed. The treatment with 1  $\mu M$  PGV-1 in HCC1954 and MDA-MB-231 cells inhibited cell movement starting 24 h of observation (Figure 2A and 2B), whereas CCA-1.1 (at the same concentration) showed a migration inhibitory effect after 48 h. However, the inhibition was not as significant as PGV-1 (Figure 2C and 2D). We observed the round-shaped cells (assumed as dead cells) presented at the end of the experiment in PGV-1-treated HCC1954 cells indicating the cells were sensitive to PGV-1. These findings suggested that PGV-1 and CCA-1.1 potentially inhibit cell migration in both HCC1954 and MDA-MB-231 cells.

### The Effect of PGV-1 and CCA-1.1 on MMP-9 and MMP-2 Expression

Cancer cell migration is correlated with its ability to degrade extracellular matrix by secreting matrix metalloproteinase enzymes (Li, *et al.*, 2017). We determined the effect of PGV-1 and CCA-1.1 on MMP-9 and MMP-2 using a gelatin zymography assay. This assay is based on the proteolytic activity



**Figure 2. The antimigratory activity of PGV-1 and CCA-1.1 in HCC1954 and MDA-MB-231 cells.**  $5 \times 10^4$  cells were seeded into IBIDI insert culture and stored for 24 h in an incubator. After cells were attached to the dish, the medium was removed and filled with starvation medium (contained 0.5% FCS) for 18 h, followed by pretreatment of 10  $\mu$ g/mL Mitomycin C and incubated for the next 2 h before being replaced with tested compound. The cell migration was observed and documented at indicated intervals (24, 42, and 48 h) through the inverted microscope (total magnification was 200x)—the morphological cells migration from treatment in (A) HCC1954 and (B) MDA-MB-231 cells. The cell-free area was determined for the area using ImageJ software and converted as the percentage of closure in HCC1954 (C) and MDA-MB-231 (D) cells. The data is displayed as the average of 3 data  $\pm$ SE, while the asterisk represents the significance value (ns=not significant; \*= $p < 0.05$ ; \*\*= $p < 0.01$ ).



**Figure 3. PGV-1 and CCA-1.1 treatment affect matrix metalloproteinase (MMP) secretion.** Cells were treated with the tested compound in the starvation medium for 24 h, then collected in the medium and used as lysate for gelatin zymography as mentioned in the method. The transparent area represented the activity of gelatinolytic MMP-2 or MMP-9 for gelatin degradation. (A) The scanned gel displayed MMP-2 secretion from treatment in HCC1954 cells. (B) The MMP-2 band was semi-quantified with ImageJ and presented as a fold of untreated. (C) The representative zymogram from MDA-MB-231 medium lysates. (D) The secretion level MMP-9 (as fold of untreated) that analyzed using ImageJ. The data are presented as a mean of 3 data  $\pm$  SD from independent experiments. The asterisk represented the significance value (ns=not significant; \* $=p<0.05$ ; \*\* $=p<0.01$ ).

of MMP to degrade gelatin (the substrate of MMP-2 or MMP-9). Our data showed that in HCC1954-medium lysates, MMP-9 could not be observed (Figures 3A). Moreover, PGV-1 and CCA-1.1 did not significantly suppress the MMP-2 expression level (Figures 3A and 3B). On the contrary, in MDA-MB-231-medium lysates, we only observed MMP-9 but not MMP-2 (Figure 3C). The MMP-9 expression level was significantly decreased in PGV-1 and CCA-1.1 treated cells, with a significance value of  $p<0.01$  and  $p<0.05$ , respectively (Figure 3C and 3D).

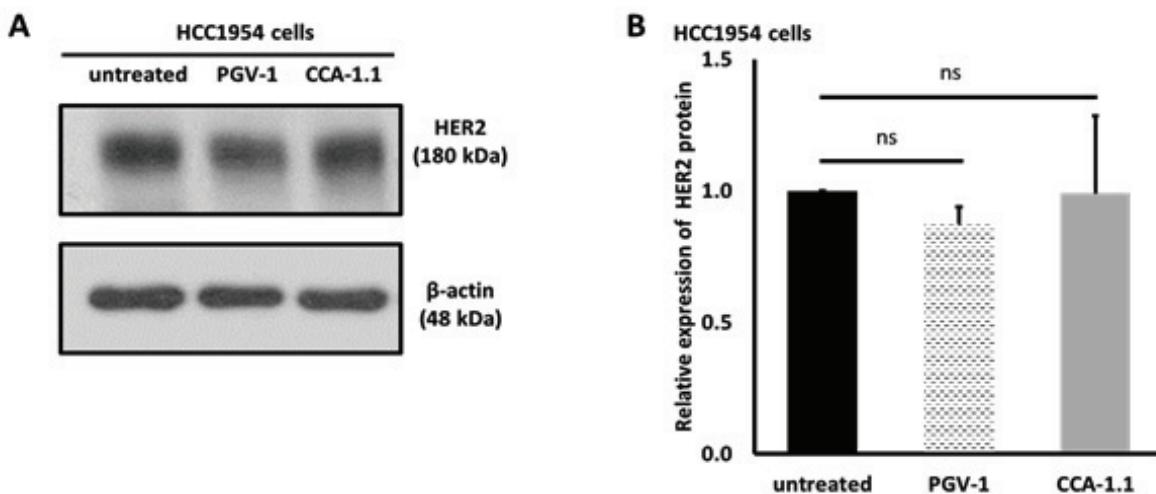
### The Effect of Curcumin Analogs PGV-1 and CCA-1.1 on HER2 Expression

Breast cancer with HER2 amplification displays a poor prognosis due to the malignancy that permits migration and invasion (Appert-Collin, et al., 2015). The known molecular mechanism of HER2-induced migration and metastasis is through activation of p120 and Rac1 (Johnson, et al., 2010). Therefore, we explored the effect of PGV-1 and CCA-1.1 on HER2 expression. A previous

report using a transfected HER2 (MCF7/HER2) revealed that PGV-1 did not interfere with HER2 expression or localization (Meiyanto, et al., 2021). In this study, we utilized HER2-endogenous HCC1954 cells and treated them with PGV-1 or CCA-1.1 to observe HER-2 expression levels by immunoblot assay (Figure 4A). PGV-1 tended to inhibit HER2 protein expression, however, the inhibition was not significant. Whereas CCA-1.1 did not affect HER2 expression (Figure 4B).

## DISCUSSION

Earlier studies have addressed the anti-proliferative effects of PGV-1 (Hermawan, et al., 2011; Meiyanto, et al., 2021, 2019), and CCA-1.1 (Novitasari, et al., 2021b, 2021c; Wulandari, et al., 2020) in breast cancer cells. Our study confirmed the cytotoxic activity of these curcumin analogs against HCC1954 and MDA-MB-231 breast cancer cells, which are defined as HER2-positive and TNBC, respectively. Previously, we reported similar cytotoxic potency of these compounds in the



**Figure 4. The effect of curcumin analogs on HER2 expression level in HCC1954 cells.** Cells were treated with either PGV-1 or CCA-1.1 for 24 h before being collected for the whole cell lysate and loaded into the immunoblot assay described in the method. (A) The representative figures of HER2 and β-actin (as loading control) from the immunoblot assay. (B) The quantification from HER2 relative expression normalized by β-actin is determined by ImageJ. The data are presented as a mean of 3 data  $\pm$  SD from independent experiments (ns=not significant).

same characteristic of breast cancer cells but with different cell lines as a model (4T1 and MCF7/HER2 cells) (Novitasari, *et al.*, 2021a). The current study also used a different approach to determine the cytotoxic (the prior study used the MTT method). Overall, these findings conclude that PGV-1 and CCA-1.1 are potent to breast cancer cells despite distinct intrinsic molecular types.

In addition to the high heterogeneity as one of the critical features in HER2-overexpressed and TNBC, both subsets are known for having the capability to migrate and invade other tissues resulting in poor prognosis (Marra, *et al.*, 2020; Schettini and Prat, 2021). Therefore, we focused on the activity of these compounds against cell migration and its correlated regulation. Our data showed that PGV-1 was more sensitive to hindering cell migration of HER2-positive cells rather than the TNBC cells. This finding is coherent with the preceding study by Meiyanto, *et al.* (2021, 2019), which reported that PGV-1 inhibits migration in MCF7/HER2 but relatively not in TNBC 4T1 cells. Moreover, our present study also confirmed that PGV-1 suppressed MMP-9 expression levels but not MMP-2. In another context, CCA-1.1 decreased MMP-9 levels but it did not alter MMP-2 protein expression. Furthermore, the absence of MMP-9 (in HCC1954 cells) or MMP-2 (in MDA-MB-231 cells) in zymograms could be due to inadequate concentration to be detectable by gelatin zymography. In a study by Yousef, *et al.* (2014) that analyzed the transcription level of MMP-9 across breast tumor subtypes, the expression of MMP-9 was elevated in basal-like MDA-MB-231 cells, while in HER2-positive HCC1954, the level was low. Furthermore, the mRNA level of MMP-2 was found to be low and the zymogram using MDA-MB-231 lysate did not show an MMP-2 band based on previous work by Kim, *et al.* (2016). Considering these reasons, deepening molecular mechanisms which differ the response using different approaches may be worthwhile for future studies.

It is widely known that cell migration is an essential step in the metastatic dissemination of breast cancer, and this development involves dramatic physiological changes in the tumor microenvironment to facilitate cancer cell movement. This complex process is regulated by numerous pathways that signal various activation that contributes to breast cancer metastasis (Li, *et al.*, 2017). From this perspective, breast cancer with overamplification of HER2 protein is believed to enhance the signal for proliferation and metastasis, thus the curcumin derivatives are hypothesized to inhibit HER2 expression at the protein level. We revealed that PGV-1 inhibited endogenous HER2 expression, in contrast with CCA-1.1, which displayed an unchanged level of HER2 compared with untreated cells. It is not a surprise in the case of PGV-1, which presented a similar result in the prior report (Meiyanto, *et al.*, 2021), but this is the first reported evidence for CCA-1.1 treatment on HER2 expression in breast cancer. Several reports have proven that these curcumin analogs inhibit various cancer cells proliferation after 24 h of treatment which is mediated through cell cycle arrest and a prolonged incubation with these compounds causes apoptosis (Lestari, *et al.*, 2019; Meiyanto, *et al.*, 2019; Novitasari, *et al.*, 2021a). Therefore, we suggest the same mechanism is contributed to the antiproliferative effect of PGV-1 and CCA-1.1 on HCC1954 and MDA-MB-231. However, the nature of HER2 activation is known through autophosphorylation (Bose, *et al.*, 2006), thus, the consequence of PGV-1 or CCA-1.1 treatment in longer incubation time toward HER2 activation will be needed for future exploration in HER2-driven cancer cells.

Despite differing by one functional group, the biological activities give varying cellular effects. We noticed that PGV-1 treatment is more potent than CCA-1.1 to inhibit HCC1954 and MDA-MB-231 cell growth. Furthermore, PGV-1 capability to inhibit cell migration and correlated proteins is also greater. Although these compounds

had similar anticancer activities in breast cancer cells, with various mechanisms of action, including cell cycle arrest, senescence induction, and ROS generation (Novitasari, *et al.*, 2021a, 2021b), the different potency in the cancer cell migration can be partly explained due to the binding interaction of the compounds with the target proteins. Molecular studies by Utomo, *et al.* (2022) and Meiyanto, *et al.* (2022) reported similar docking scores between PGV-1 and CCA-1.1 into several putative markers, including ROS-metabolizing enzymes (NQO1, NQO2, and GLO1) and mitotic regulators (CDK1, Aurora A, KIF11, and WEE1). A similar study on the protein regulator of cancer cell metastasis is worth exploring. This study would raise another consideration for pharmaceutically developing PGV-1 and CCA-1.1 as a chemotherapeutic for breast cancer, particularly aggressive ones.

## CONCLUSION

Curcumin analogs PGV-1 and CCA-1.1 suppress MMP-9 secretion, and PGV-1 significantly inhibits cell migration. The HER2 protein expression is not affected by PGV-1 or CCA-1.1 treatment in HER2-endogenous breast cancer cells.

## ACKNOWLEDGMENT

None

## REFERENCES

Appert-Collin, A., Hubert, P., Crémel, G., and Bennasroune, A., 2015, Role of ErbB Receptors in Cancer Cell Migration and Invasion, *Frontiers in Pharmacology*, **6**.

Bose, R., Molina, H., Patterson, A.S., Bitok, J.K., Periaswamy, B., Bader, J.S., *et al.*, 2006, Phosphoproteomic analysis of Her2/neu signaling and inhibition, *PNAS*, **103**, 9773-9778.

Di, J., Huang, H., Qu, D., Tang, J., Cao, W., Lu, Z., *et al.*, 2015, Rap2B promotes proliferation, migration, and invasion of human breast cancer through calcium-related ERK1/2 signaling pathway, *Sci Rep*, **5**, 12363.

Fragomeni, S.M., Sciallis, A., and Jeruss, J.S., 2018, Molecular subtypes and local-regional control of breast cancer, *Surg Oncol Clin N Am*, **27**, 95-120.

Freudenberg, J.A., Wang, Q., Katsumata, M., Drebin, J., Nagatomo, I., and Greene, M.I., 2009, The role of HER2 in early breast cancer metastasis and the origins of resistance to HER2-targeted therapies, *Exp Mol Pathol*, **87**, 1-11.

Grada, A., Otero-Vinas, M., Prieto-Castrillo, F., Obagi, Z., and Falanga, V., 2017, Research Techniques Made Simple: Analysis of Collective Cell Migration Using the Wound Healing Assay, *Journal of Investigative Dermatology*, **137**, e11-e16.

Hermawan, A., Fitriasari, A., Junedi, S., Ikawati, M., Haryanti, S., Widaryanti, B., *et al.*, 2011, PGV-0 and PGV-1 increased apoptosis induction of doxorubicin on MCF-7 breast cancer cells, *Pharmacon*, **12**, 55-59.

Johnson, E., Seachrist, D.D., DeLeon-Rodriguez, C.M., Lozada, K.L., Miedler, J., Abdul-Karim, F.W., and Keri, R.A., 2010, HER2/ErbB2-induced Breast Cancer Cell Migration and Invasion Require p120 Catenin Activation of Rac1 and Cdc42, *J Biol Chem*, **285**, 29491-29501.

Kim, S., Lee, J., Jeon, M., Lee, J.E., and Nam, S.J., 2016, Zerumbone suppresses the motility and tumorigenecity of triple negative breast cancer cells via the inhibition of TGF- $\beta$ 1 signaling pathway, *Oncotarget*, **7**, 1544-1558.

Kolditz, F., Krausze, J., Heinz, D.W., Niemann, H.H., and Müller-Goymann, C.C., 2014, Wound healing potential of a dimeric InlB variant analyzed by *in vitro* experiments on re-epithelialization of human skin models, *European Journal of Pharmaceutics*

and Biopharmaceutics, Theme Issue: Dermal and Transdermal Drug Delivery, **86**, 277-283.

Lestari, B., Nakamae, I., Yoneda-Kato, N., Morimoto, T., Kanaya, S., Yokoyama, T., *et al.*, 2019, Pentagamavunon-1 (PGV-1) inhibits ROS metabolic enzymes and suppresses tumor cell growth by inducing M phase (prometaphase) arrest and cell senescence, *Sci Rep*, **9**, 1-12.

Li, H., Qiu, Z., Li, F., and Wang, C., 2017, The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis, *Oncol Lett*, **14**, 5865-5870.

Lv, Y., Zhao, X., Zhu, L., Li, S., Xiao, Q., He, W., and Yin, L., 2018, Targeting intracellular MMPs efficiently inhibits tumor metastasis and angiogenesis, *Theranostics*, **8**, 2830-2845.

Marra, A., Trapani, D., Viale, G., Criscitiello, C., and Curigliano, G., 2020, Practical classification of triple-negative breast cancer: intratumoral heterogeneity, mechanisms of drug resistance, and novel therapies, *NPJ Breast Cancer*, **6**, 1-16.

Meiyanto, E., Husnaa, U., Kastian, R.F., Putri, H., Larasati, Y.A., Khumaira, A., *et al.*, 2021, The Target Differences of Anti-Tumorigenesis Potential of Curcumin and its Analogues Against HER-2 Positive and Triple-Negative Breast Cancer Cells, *Adv Pharm Bull*, **11**, 188-196.

Meiyanto, E., Novitasari, D., Utomo, R.Y., Susidarti, R.A., Putri, D.D.P., and Kato, J., 2022, Bioinformatic and Molecular Interaction Studies Uncover That CCA-1.1 and PGV-1 Differentially Target Mitotic Regulatory Protein and Have a Synergistic Effect against Leukemia Cells, *Indonesian Journal of Pharmacy*, **33**(2), 225-233.

Meiyanto, E., Putri, H., Larasati, Y.A., Utomo, R.Y., Jenie, R.I., Ikawati, M., *et al.*, 2019, Anti-proliferative and Anti-Metastatic Potential of Curcumin Analogue, Pentagamavunon-1 (PGV-1), Toward Highly Metastatic Breast Cancer Cells in Correlation With ROS Generation, *Adv Pharm Bull*, **9**, 445-452.

Novitasari, D., Handayani, S., and Jenie, R.I., 2018, Ethanolic Extract of Hedyotis corymbosa L. Inhibits Migration and MMP-9 Activity on Metastatic Breast Cancer Cells, *Indonesian Journal of Cancer Chemoprevention*, **9**, 16-22.

Novitasari, D., Jenie, R.I., Utomo, R.Y., Kato, J.Y., and Meiyanto, E., 2021a, CCA-1.1, a Novel Curcumin Analog, Exerts Cytotoxic anti-Migratory Activity toward TNBC and HER2-Enriched Breast Cancer Cells, *Asian Pac J Cancer Prev*, **22**, 1827-1836.

Novitasari, D., Jenie, R.I., Wulandari, F., Putri, D.D.P., Kato, J., and Meiyanto, E., 2021b, A Curcumin Like Structure (CCA-1.1) Induces Permanent Mitotic Arrest (Senescence) on Triple Negative Breast Cancer (TNBC) Cells, *4T1, Res J Pharm Technol*, **14**, 4375-4382.

Novitasari, D., Wulandari, F., Jenie, R.I., Utomo, R.Y., Kato, J.-Y., and Meiyanto, E., 2021c, A new curcumin analog, CCA-1.1, induces cell cycle arrest and senescence toward ER-positive breast cancer cells, *International Journal of Pharmaceutical Research*, **13**, 1-9.

Rakha, E.A., and Chan, S., 2011, Metastatic Triple-negative Breast Cancer, *Clinical Oncology*, **23**, 587-600.

Sangrar, W., Shi, C., Mullins, G., LeBrun, D., Ingalls, B., and Greer, P.A., 2015, Amplified Ras-MAPK signal states correlate with accelerated EGFR internalization, cytostasis and delayed HER2 tumor onset in Fer-deficient model systems, *Oncogene*, **34**, 4109-4117.

Schettini, F. and Prat, A., 2021, Dissecting the biological heterogeneity of HER2-positive breast cancer, *Breast*, **59**, 339-350.

Turashvili, G., and Brogi, E., 2017, Tumor Heterogeneity in Breast Cancer, *Frontiers in Medicine*, **4**.

Utomo, R.Y., Wulandari, F., Novitasari, D., Lestari, B., Susidarti, R.A., Jenie, R.I., *et al.*, 2022,

Preparation and cytotoxic evaluation of PGV-1 derivative, CCA-1.1, as a new curcumin analog with improved physicochemical and pharmacological properties, *Advanced Pharmaceutical Bulletin*, 12(3), 603-612.

Wulandari, F., Ikawati, M., Novitasari, D., Kirihata, M., Kato, J., and Meiyanto, E., 2020, New curcumin analog, CCA-1.1, synergistically improves the antiproliferative effect of doxorubicin against T47D breast cancer cells, *Indonesian Journal of Pharmacy*, 31, 244-256.

Yousef, E.M., Tahir, M.R., St-Pierre, Y., and Gaboury, L.A., 2014, MMP-9 expression varies according to molecular subtypes of breast cancer, *BMC Cancer*, 14, 609.

Zhang, W., Wang, F., Xu, P., Miao, C., Zeng, X., Cui, X., et al., 2014, Perfluorooctanoic acid stimulates breast cancer cells invasion and up-regulates matrix metalloproteinase-2/-9 expression mediated by activating NF- $\kappa$ B, *Toxicol Lett*, 229, 118-125.